Supplementary material:

Table S1: Database details and accession numbers of retromer protein homologues identified in this study. Databases used for BLAST analysis for the species studied are given, as well as accession numbers for protein orthologues identified for SNX (sorting nexins), Vps26, Vps29, Vps35, and Vps10, shown schematically in Figures 1 and 5, and used for the phylogenetic analysis in Figures 2, 3, 4, 6 and 8. Vps26 ORFs in *Monosiga brevicollis* and *Tetrahymena thermophila* are probably mis-annotated, resulting in predicted Vps26 polyeptide of >2000 residues (Vps26 is typically ~300 residues). Similarly, Vps29 ORFs of *Monosiga brevicollis* and *Naegleria gruberi*, encode products of >890aa, compared to the typical size for Vps29 of approximately 190 aa. Only one of the *Nematostella vectensis* Vps35 sequences was included in the phylogenetic analysis, because the other was abnormally short, i.e. 193 aa versus the typical size for Vps35 of approximately 790 aa.

Table S2: Details of manual BLAST and HMMer searches for all cargo (excluding Vps10) as well as GNPTAB and NAGPA, shown in Figure 9. Columns listed by organism, database identifier of homologue, E-value using the query, and E-value for reverse BLAST against human genome.

Figure S1: Phylogenetic reconstruction of the SNX protein family across the Fungi. Tree shown is the best Bayesian topology. Numerical values at the nodes of the tree (x/y/z) indicate statistical support for MrBayes/PhyML/RAxML (posterior probability/bootstrap/bootstrap respectively). Values for highly supported nodes have been replaced by symbols as indicated.

Figure S2: TbVps5 is a BAR-domain protein. Panel A: JOY alignment (Mizuguchi et al., 1998) showing a comparison of the environments of residues of the modelled structure (TbVps5) with those of the homologous protein (2raia). 2raia: chain a, of human SNX9, corresponding to the orange-coloured monomer of the dimer shown in Figure 7A. TbVps5: chain a, of TbVps5, corresponding to the green-coloured monomer of the dimer shown in Figure 7B. Panel B: Ramachandran plot of the modelled structure of TbVps5 showing that 99.6% of the residues in the model fall within the allowed regions. Panel C: analysis of conserved residues in the SNX9 structure and the TbVps5 dimer model, which are involved in dimerisation.

Figure S3: The *T. brucei* retromer complex is required for viability in the bloodstream stage. Panel A: qRT-PCR to confirm BSF/PCF upregulation for all retromer components in *T. brucei* at the mRNA level. Panel B: Growth curves following RNAi induction for TbVps5, TbVps26, and TbVps35. Panel C: qRT-PCR to confirm knockdown after RNAi induction for TbVps5, TbVps26, and TbVps35. Panel D: Quantitative analysis of copy numbers of nuclei and kinetoplasts following RNAi induction for TbVps5, TbVps26, and TbVps35. A modest disruption in cell cycle progression (increase in 2N2K post mitotic cells) was observed after RNAi induction for TbVps5. For TbVps35 >2N2K cells accumulated after RNAi induction. Results in Panels A-D are the combined data from at least duplicate biological replicates. Error bars show standard deviation. Panel E: Examples of >2N2K cells, seen after RNAi induction for TbVps35, showing multiple distinct nuclei and kinetoplasts in enlarged undivided cells. Scale bar is 2 μm

Movie S1: Movie of TbVps5 dimer model. The two monomers of TbVps5 are coloured green and cyan. The dimer adopts a curved shape characteristic of BAR domain dimers, with extended helices at the ends of the curved structure compared to SNX9, rotated along the x-axis.

Movie S2: Movie of rotating TbVps5 model. Superimposed structures for human SNX9 dimer (orange) and TbVps5 dimer (green) rotated along the x axis, showing the concave face of the curved structure with the PX domains extending above and below.